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Neolecta vitellina, first record from Romania, with notes on habitat and phenology

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Abstract — *Neolecta vitellina,* one of the rarely collected ascomycete species in Europe, is reported from Romania for the first time. The species was found on the ground, under Norway spruce (*Picea abies*) in 2004, from the Nature Reserve "Tinovul Mare" Poiana Stampei (Eastern Carpathians, Romania). Subsequent field observations have confirmed the presence of *Neolecta vitellina* in the same location, in the period 2005–10. A description and photographs of the specimens are presented.

Key words — *Ascomycota, Neolectaceae,* ITS sequence

Introduction

The genus *Neolecta* Speg. belongs to the ascomycete family *Neolectaceae* (Redhead 1977), order *Neolectales* (Landvik et al. 1993), class *Neolectomycetes* (*Taphrinomycotina, Ascomycota*). This genus includes three accepted species —*N. flavovirescens* Speg., *N. irregularis* (Peck) Korf & J.K. Rogers, *N. vitellina*— with clavate, unbranched to lobed yellow ascomata, up to about 7 cm tall (Landvik et al. 2003). Of these three species, only *N. vitellina* has been reported from Europe (Bresadola 1882, Geitler 1958, Hansen & Knudsen 2000: 48–50, Krieglsteiner 1993: 421, Landvik et al. 2003, Ohenoja 1975, Redhead 1989). Work published by European researchers about *Neolecta vitellina* refers mainly to phylogeny, morphology and ultrastructure (Landvik 1996, 1998; Landvik et al. 1993, 2001, 2003). The purpose of this paper is to report the presence of *Neolecta vitellina* in Romania and to provide morphological and ecological notes on this fungus.

Materials & methods

During 2005–2010 the known habitat of *Neolecta vitellina* in the Nature Reserve "Tinovul Mare" Poiana Stampei (Eastern Carpathians, Romania) was monitored for

Table 1. List of *Neolecta vitellina* specimens, GenBank (ITS) accession numbers, and sequence identity compared with the Romanian specimen.

Herbarium: Voucher No.	Accession No.	Sequence identity with FJ171855.1	Origin	Date; Collector(s)
I 114506	FJ171855.1	N/A	Romania	2 July 2005; VC Chinan
FH:DAH-7	FJ171850.1	99%	Norway, near Oslo	14 Aug 2002; S Landvik, DA Hewitt, P Inderbitzin, S Huhtinen, inter al.
FH:DAH-18	FJ171849.1	99%	USA, MA	3 Nov 2005; DA Hewitt, B Wolfe
FH:DAH-22	FJ171847.1	99%	USA, MA	15 Nov 2005; WJ Neill
FH:DAH-21A	FJ171852.1	99%	USA, MA	17 Nov 2005; DA Hewitt
FH:DAH-21B	FJ171853.1	99%	USA, MA	17 Nov 2005; DA Hewitt
FH:DAH-11	FJ171851.1	99%	USA, MA	6 Nov 2003; DA Hewitt, G Riner
FH:KH.04.34	FJ171848.1	100%	USA, NM	30 Aug 2004; K Hansen, B Perry
OSC:119159	FJ171854.1	99%	USA, OR	3 Oct 2001; M Russell

ascomata. Fresh specimens were photographed in situ prior to collection. Ascomata were sectioned and mounted in Melzer's reagent for observation of asci and ascospores under the light microscope.

The ITS DNA sequence was generated from a dried *Neolecta vitellina* ascoma (I 114506) that was crushed using a FASTPREP DNA extraction machine (Qbiogene, Inc.). DNA was extracted from crushed material using a phenol-chloroform extraction protocol. The ITS (Internal Transcribed Spacer) region was amplified by PCR using primers ITS4 and ITS5 (http://www.biology.duke.edu/fungi/mycolab/primers.htm). We used BlastN (Altschul et al. 1997) to query the GenBank database for similar sequences and calculate percent identity and gaps. The obtained ITS sequence is deposited in GenBank (accession number FJ171855.1).

Analyzed specimens are deposited in the Herbarium of Alexandru Ioan Cuza University, Faculty of Biology, Iaşi, Romania (I) and Farlow Herbarium, Harvard University, Cambridge, USA (FH).

Taxonomy

Neolecta vitellina (Bres.) Korf & J.K. Rogers, Phytologia 21: 204 (1971). Fig. 1

≡ *Geoglossum vitellinum* Bres., Rev. Mycol. 4: 212 (1882).

Ascomata 20–40 mm long, irregularly clavate, lanceolate or spathulate and consisting of a sterile zone (stipe) at the bottom and a fertile zone (hymenium)

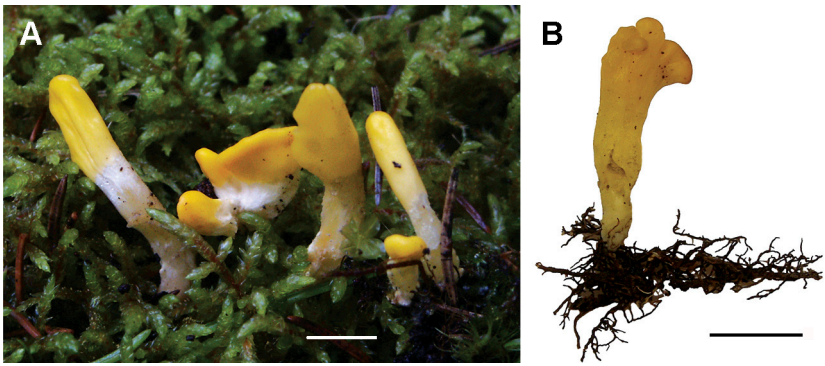

Figure 1. *Neolecta vitellina*.
A-ascomata in habitat; B-ascoma attached to a root of Norway spruce (*Picea abies*).
Scale bar = 1 cm.

on the top; fertile zone 9–18 mm long, 3–8 mm wide, yellow to bright yellowish, smooth, sometimes longitudinally subplicate, with margin entire or slightly irregularly lobate; sterile zone 10–22 mm long, 2–4 mm wide at the fertile zone, pubescent or tomentose, whitish or pale yellow (Fig. 1A).

Asci 55–75 µm long, 4–5.5 µm wide above, 3–3.5 µm wide below, cylindrical to cylindrical-clavate, 8-spored. Paraphyses absent. Ascospores uniseriate, unicellular, 5.5–8 × 3–4 µm, reniform, ellipsoid or ovoid, hyaline, smooth. Phialoconidia not found associated with ascospores.

Habitat on the ground, among mosses, in coniferous forest under Norway spruce (*Picea abies* (L.) H. Karst.) at an altitude of 915 m.

Sequence analysis BlastN searches of GenBank with the ITS sequence (646 nucleotides) of the Romanian *N. vitellina* specimen (I 114506) produced significant alignments with ITS sequences from *N. vitellina* specimens collected from Norway and USA (Table 1).

Specimens examined: **ROMANIA**. Eastern Carpathians: Nature Reserve "Tinovul Mare" Poiana Stampei, 47°18′02.69″N, 25°06′45.59″E, 915 m altitude, on ground under *Picea abies*, 4 October 2004, leg. Chinan (I 114507); 2 July 2005, leg. Chinan (I 114506, FH:DAH-31, GenBank ITS— FJ171855.1); 29 June 2006, leg. Chinan (I 114508); 30 June 2007, leg. Chinan (I 137103); 12 July 2008, leg. Chinan (I 137104); 8 July 2010, leg. Chinan (I 137105).

Discussion

Neolecta vitellina is rarely collected in Europe, where it has a boreal-montane distribution. The species has previously been reported in Norway, Sweden, Finland (Hansen & Knudsen 2000), and Italy (Bresadola 1882, Geitler 1958). In Romania, *N. vitellina* was found in the northern part of the country at the periphery of "Tinovul Mare" Poiana Stampei peat bog on the ground among

mosses under Norway spruce (*Picea abies*). Our report represents the first record of this species for Romania and the Carpathian Mountains.

Two ascomata were first found on October 2004. In July 2005, more specimens were in the same location with 53 ascomata inventoried in approximately a 10 m^2 area. Subsequent monitoring during 2006–10 has confirmed its presence in the same place and shown that late June and early July seem to be the optimal time for *Neolecta vitellina* to form ascomata in Romania. For comparison, Landvik et al. (2003) reported *N. vitellina* from Northern Europe in August while Redhead (1977) reports the species from North America in August–October. Additionally, a Bresadola collection of *Geoglossum vitellinum* from Southern Tyrol (Italy) in the Patouillard collection at the Farlow Herbarium is dated 'Aug. 1889', later than the fruiting time recorded herein for the Romanian populations.

The macroscopic and microscopic characters of the specimens collected from Romania are in accordance with the literature (Redhead 1977, Hansen & Knudsen 2000, Landvik 2003) and the ITS sequence derived from one ascoma (I 114506) matches those of other specimens identified as *Neolecta vitellina*.

We also confirm that *Neolecta vitellina* ascomata are attached to Norway spruce roots (Fig. 1B), on which was observed a whitish sleeve consisting of mycelium. Regarding this aspect, Redhead (1979) mentions that *Neolecta vitellina* is possibly a root parasite, based on morphology and the close association of the hyphae and the roots to which the hyphae are attached.

The presence of this species in the Nature Reserve "Tinovul Mare" Poiana Stampei, part of the European Ecological Natura 2000 Network, emphasizes the importance of this site for macrofungi conservation.

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